Research Letter

Endogenous Hydrogen Sulfide Overproduction in Down Syndrome

To the Editor:

The cystathionine beta synthase (CBS) gene is localized on chromosome 21 (21q22.3). This enzyme is one of three enzymes able to produce hydrogen sulfide. CBS is overexpressed in Down syndrome with levels 166% of normal values in fibroblasts [Chadeaufx et al., 1985] and 1,200% in myeloblasts [Taub et al., 1999]. The CBS overexpression could induce an overproduction of hydrogen sulfide in Down syndrome patients, and this overproduction is potentially able to induce some of the clinical signs of Down syndrome such as hypotonia and mental retardation. As thiosulfate is the main catabolite of hydrogen sulfide [Kangas and Savolainen, 1987], we compared the levels of this molecule in the urine of Down syndrome patients and control subjects. Human erythrocytes contain various forms of hemoglobin. These include sulfhemoglobin, which is formed by transformation of the ferric derivative of hemoglobin, methemoglobin. Sulfhemoglobin production requires hydrogen sulfide (or another sulfide) and methemoglobin [Nichols et al., 1968]. The determination of sulfhemoglobin in erythrocytes was therefore also used to assess hydrogen sulfide production in Down syndrome.

Informed consent was obtained from Down syndrome patients and their parents and from controls. The subjects were assigned to three groups. Group 1 (diet-matched pairs) consisted of 21 pairs of subjects (17 of them were included in a previously published study [Belardinelli et al., 2001]. In each volunteer family, one Down syndrome subject and one relative (mother or father in most families, brother or sister in rare cases) were given identical diets. This group consisted of 13 male and 8 female Down syndrome subjects and matched controls (10 male and 11 female). Sulfur compounds were excluded from the daily treatments of Down syndrome patients and controls. Group 2 consisted of 30 patients with Down syndrome (19 male and 11 female) and 20 controls (volunteers from the laboratory; 10 male and 10 female). In this group, age distribution was similar for Down syndrome patients and controls (Table I). The first urine produced in the morning was collected from the subjects of groups 1 and 2 in vials containing boric acid used as a preservative. Thiosulfate was determined in urine by colorimetry after chromatographic separation [Voroteliak et al., 1993]. Creatinine was determined by the manual Jaffe method. Group 3 consisted of 60 Down syndrome patients (33 male and 27 female) and 60 age-matched controls (35 male and 25 female). Venous blood was withdrawn in fasting subjects of group 3; erythrocytes were separated by centrifugation and hemolysates were frozen until use. Sulfhemoglobin was determined by spectrophotometry and the results are expressed as ratio of absorbance (A) at various wavelengths: (A622 nm – A636 nm)/(A535 nm + A560 nm) 0.5 × 10². This ratio was used because it is not affected by differences between the respective concentrations of oxygenated and unoxgenated hemoglobin.

A significant difference was observed in the urinary excretion of thiosulfate between Down syndrome patients and relatives of group 1 (diet-matched pairs) (Table I). In group 2, statistical analysis indicates that the differences in thiosulfate excretion persisted. To confirm that hydrogen sulfide was overproduced in Down syndrome patients, we studied erythrocyte sulfhemoglobin content in subjects of group 3. The wavelength ratios were 2.51 ± 0.04 and 2.00 ± 0.08 (SEM) for patients with Down syndrome and controls, respectively (P < 0.001).

We obtained two different types of evidence for the overproduction of hydrogen sulfide in Down syndrome patients. The main function of CBS is to catalyze the first step of transsulphuration pathway, producing cysteine from homocysteine. In vivo, the high level of CBS activity in Down syndrome results in low concentrations of the substrate of CBS (homocysteine) in plasma [Chadeaufx et al., 1988]. CBS also has another enzymatic activity: the production of hydrogen sulfide from cysteine [Stipanuck and Beck, 1982]. The endogenous production of hydrogen sulfide can be estimated by monitoring thiosulfate excretion in urine (31 μmoles/day in control adults) [Sorbo and Ohman, 1978]. After hydrogen sulfide poisoning, the excretion of thiosulfate in urine increased significantly [Kangas and Savolainen, 1987].
Clinical and biological observations have established a relationship between Down syndrome and chronic hydrogen sulfide poisoning. First, significant reductions in sensory nerve conduction velocity have been observed in workers exposed to carbide disulfide [Takebayashi et al., 1988] and in Down syndrome patients [Christensen et al., 1988]. Secondly, impaired color discrimination was found to be significantly more frequent in workers chronically exposed to carbide disulfide than in controls [Raitta et al., 1981; Vanhoorne et al., 1996] and Down syndrome patients have also been shown to have significant defects in color vision and low-contrast sensitivity [Rocco et al., 1977; Perez-Carpinell et al., 1994]. Some studies have suggested that endogenous hydrogen sulfide has a physiological function, as a smooth muscle relaxant acting in synergy with nitric oxide, or as an endogenous neuromodulator, inducing long-term hippocampal potentiation and increasing NMDA receptor activity [Abe and Kimura, 1996; Hosoki et al., 1994]. The overproduction of hydrogen sulfide in Down syndrome patients may therefore cause muscle (hypotonia) and brain (mental retardation) dysfunction. Sodium nitrite has been used to treat acute hydrogen sulfide poisoning. The administration of 4 mg/kg sodium nitrite to volunteers resulted in up to 7% methemoglobin [Kiese and Weger, 1969]. A lower dose of nitrite (or nitrate as nitrite precursor) could be used to treat orally the chronic hydrogen sulfide overproduction of patients with Down syndrome.

REFERENCES


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